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14. ABSTRACT
Androgens are hormones that play a critical role in stimulating prostate cancer growth. Androgens activate a protein called the androgen receptor (AR), which regulates genes involved in cell growth. Although powerful anti-androgen drugs can be administered to block AR action and have been used successfully to treat patients with prostate cancer, over time the tumors become resistant to the drugs, leaving few treatment options. The goal of this proposal is to develop a new approach to block AR activity and stop prostate cancer growth using a new family of molecules called multivalent peptidomimetic conjugates. To accomplish our goals, we will create a set of conjugates with anti-androgens linked to the peptidomimetic backbone at variable intervals along the molecular chain. We will test these molecules for their ability to bind to AR. Those that bind tightly will then be tested in tumor models to evaluate if they block androgen-dependent prostate cancer cell growth. To understand how these molecules block AR function, we will determine the three-dimensional structure of AR bound to the peptidomimetic conjugates. These studies will be used to guide our ability to tailor the conjugates for optimal interactions with the AR.

15. SUBJECT TERMS
androgen receptor, prostate cancer, peptidomimetic conjugates,

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TABLE OF CONTENTS: **page no**

1. **Introduction.....1**
2. **Keywords.....1**
3. **Accomplishments.....1**
4. **Impact.....3**
5. **Changes/Problems.....3**
6. **Products.....3**
7. **Participants & Other Collaborating Organizations.....4**
8. **Special Reporting Requirements.....5**
9. **Appendices.....5**

1. INTRODUCTION:

Androgens are hormones that play a critical role in stimulating prostate cancer growth. Androgens activate a protein called the androgen receptor (AR), which regulates genes involved in cell growth. Although powerful anti-androgen drugs can be administered to block AR action and have been used successfully to treat patients with prostate cancer, over time the tumors become resistant to the drugs, leaving few treatment options. The goal of this proposal is to develop a new approach to block AR activity and stop prostate cancer growth using a new family of molecules called multivalent peptidomimetic conjugates. To accomplish our goals, we are creating a set of conjugates with anti-androgens linked to the peptidomimetic backbone at variable intervals along the molecular chain. We are testing these molecules for their ability to bind to AR. Those that bind tightly are being tested in tumor models to evaluate if they block androgen-dependent prostate cancer cell growth. To understand how these molecules block AR function, we will determine the three-dimensional structure of AR bound to the peptidomimetic conjugates. These studies will be used to guide our ability to tailor the conjugates for optimal interactions with the AR.

2. KEYWORDS:

KEYWORDS: Androgen receptor, prostate cancer, peptidomimetic conjugates,

3. ACCOMPLISHMENTS:

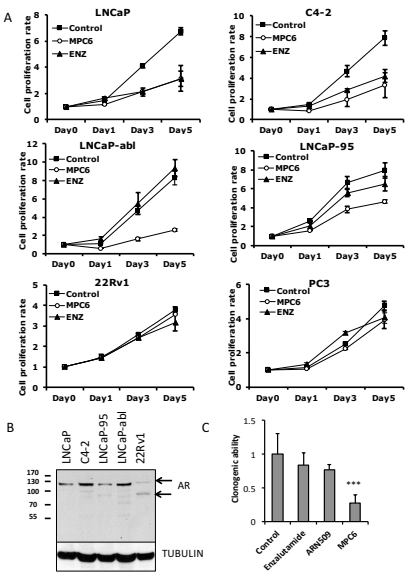
- What were the major goals and objectives of the project?

The major goals of the project are:

1) Synthesize a family of multivalent peptidomimetic conjugates
2) Test peptidomimetic conjugates in a series of in vitro and cell-based assays
3) Conduct studies of pharmacological potential through *in vivo* mouse xenograft and PK/PD studies
4) Establish the mechanism of action of peptidomimetic conjugates on the Androgen Receptor through biophysical and X-ray crystallographic studies.

- What was accomplished under these goals?
- Major activities for this reporting period:

- **Major Task 1:** Synthesize a family of multivalent peptidomimetic conjugates
- Subtask 1: Design, synthesize, purify and characterize a family of peptidomimetic oligomer conjugates



We synthetized a multivalent conjugate presenting two bioactive ethisterone ligands arrayed as spatially defined pendant groups on a peptoid oligomer. The conjugate, named Multivalent Peptoid Conjugate 6 (MPC6), suppressed the proliferation of multiple AR-expressing prostate cancer cell lines including those that failed to respond to enzalutamide and ARN509 (Figure 1).

Figure 1. MPC6 inhibit proliferation of multiple AR-expressing prostate cancer cell lines.
A) AR-expressing prostate cancer cell lines (LNCaP, LNCaP-C4-2, LNCaP-abl, LNCaP-95 and 22Rv1) and AR-negative PC3 cells were treated with control or a single dose of 10 μM MPC6 for 5 days and cell growth measured. B) Western blot of AR from prostate cancer cells C) Colony formation in LNCaP-abl cells, treated with vehicle, 10 μM enzalutamide, 10 μM ARN509, or 10 μM MPC6, were measured by a clonogenic assay. ***p=0.0003.

Major Task 2: Test the peptidomimetic conjugates in a series of in vitro and cell-based assays

Subtask 2: Conduct *in vitro* activity assays

- Determine peptidomimetic conjugates ability to compete for DHT-binding to the AR LBD in vitro
- Determine peptidomimetic conjugates impact on coactivator peptide binding in vitro

Subtask 3: Conduct cell-based activity assays

- Examine the ability of the peptidomimetic conjugates to modulate AR-dependent transcriptional activity
- Determine peptidomimetic conjugates ability to promote AR-YFP nuclear localization and to block DHT-dependent AR nuclear localization
- Validate the impact of peptidomimetic conjugates on AR transcriptional activation of endogenous AR target genes by qPCR, and recruitment of AR to targets by ChIP
- Evaluate peptidomimetic conjugates ability to inhibit proliferation of therapy-resistant prostate cancer cells

We also synthesized MPC6 derivatives with diverse linkers and peptoid backbone topology and tested them in cell based proliferation assays (Figure 2A-C). We found that increased spacing between ethisterone moieties and changes in peptoid topology eliminated the anti-proliferative effect of MCP6 in therapy resistant prostate cancer, suggesting that both ethisterone ligand presentation and scaffold characteristics contribute to MPC6 activity. We also showed that compared to MPC6, the ethisterone ligand alone failed inhibit cell proliferation at the maximal concentration tested, suggesting that multivalency plays an important role in the activity of MPC6 (Figure 2D).

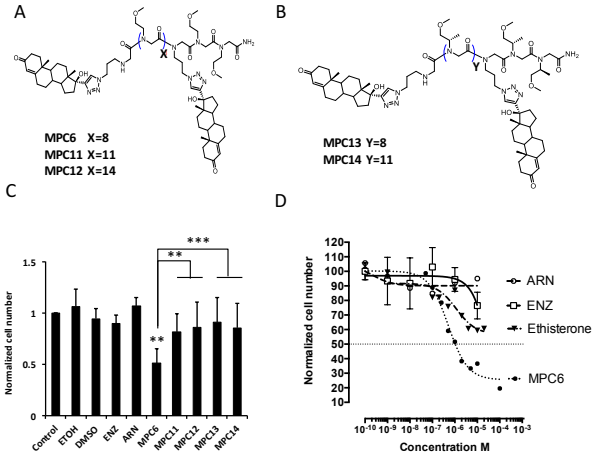


Figure 2. MPCs impact the proliferation of LNCaP-abl cells.
A) Chemical structures of linear divalent ethisterone-peptoid conjugates MPC6, MPC11 and MPC12, spaced by 8, 11 and 14 monomers, respectively. B) Chemical structures of divalent ethisterone-peptoid conjugates MPC14 and MPC15 with a methyl group on peptoid backbone. C) LNCaP-abl cells were treated with 10 µM of vehicle (Ethanol; EtOH, or DMSO), enzalutamide (ENZ), ARN509, or MPCs for 72h and cell proliferation measured. The error bar represents standard deviation from six independent experiments. **p<0.01, ***p<0.001. D) LNCaP-abl cells were treated with the indicated compounds and concentrations. Cell proliferation was measured and the EC_{50} calculated. Each point represents a mean value of three independent experiments. Error bars = standard deviation.

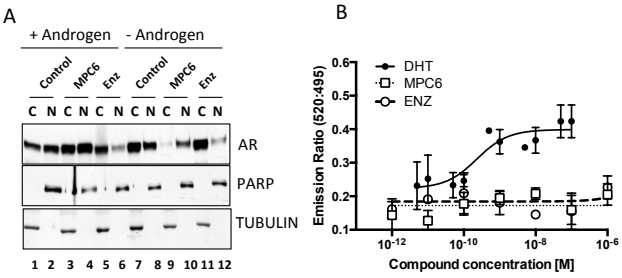


Figure 3. MPC6 promotes AR nuclear localization and blocked AR-coactivator binding
A) LNCaP-abl cells cultured in the presence or absence of androgen were treated with vehicle, 10 µM MPC6, or 10 µM enzalutamide overnight before subcellular fractionation. Western blot shows AR expression in cytoplasm (C) and nucleus (N). B) *In vitro* TR-FRET analysis of the interaction between GST-tagged AR-LBD, terbium-labeled anti-GST antibody, and fluorescein-labeled AR FxxLF co-activator peptide. Titration of DHT, enzalutamide (ENZ), and MPC6 for 10 nM DHT bound AR.

We determined that MPC6 promoted AR nuclear localization (Figure 3A). We also found that MPC6 was able to compete for coactivator peptide binding in vitro using a time resolved fluorescence resonance energy

transfer (TR-FRET) assay (Figure 3B).

We also showed that MPC6 reduced the expression of key AR target gene UBCEC2 involved in prostate cancer cell proliferation (Figure 4). These results were reported in a paper in Cancer Research 2016 Aug 3. [Epub ahead of print]

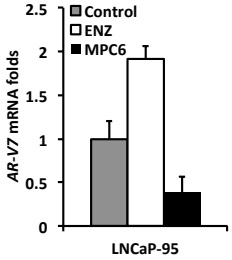


Figure 4. MPC6 decreased AR-V7 expression and AR-V7 target gene transcription in prostate cancer cells.
LNCaP-95 cells were treated with vehicle, 10 μM MPC6, or 10 μM enzalutamide for 48 hours. Total RNA was extracted, reverse transcribed and qRT-PCR performed to assess *UBE2C* mRNA expression. **p<0.01.

Major Task 4. The androgen receptor ligand binding domain is challenging to crystallize do to its misfolding and degradation in bacteria. This can be overcome by incubating a large volume of culture, >10 L, with an agonist ligand such as DHT. We added MPC6 to the fermentation media when IPTG was added to induce AR expression. We tried this under a number of different conditions and in all cases the protein was not stabilized by MPC6. We then obtained crystals with DHT, which diffracted to ~ 2 Å. These crystals were transferred to another well with the same mother liquor but with 2-5mM MPC6. We then solved a number of structures, but the DHT remained in all cases. We are now optimizing the protein expression with a new slightly shorter construct and will test if this can be stabilized by MPC6. Other plans include extended dialysis to exchange DHT for MCP6.

- **What opportunities for training and professional development did the project provide?**
  Nothing to Report."

- **How were the results disseminated to communities of interest?**
  We published a paper on this topic in Cancer Research Cancer Res. 2016 Sep 1;76(17):5124-32. doi: 10.1158/0008-5472.CAN-16-0385. Epub 2016 Aug 3, which is widely read by basic and clinical oncologists. The study was also highlighted in the journal *Nature Reviews Urology*, which targets Urologists in clinical practice.

- **What do you plan to do during the next reporting period to accomplish the goals and objectives?**

  We are planning to synthesize oligomer conjugates bearing heterogeneous ligand displays (incorporating both ethisterone and flufenamic acid as pendant groups targeting distinct regions of the androgen receptor) and to synthesize derivatives of MPC6 that include cationic side chains to improve efficacy. We will test these in in vitro assays and cell-based models to test their effect on cell proliferation of therapy resistant prostate cancer. We also plan DMPK and xenograft studies to determine the impact of MPC6 on therapy resistant prostate cancer cell *in vivo*.

4. IMPACT:

Our work describes the biological evaluation of a new set of multivalent peptoid conjugates (MPCs) We are discovering the therapeutic potential of an innovative new family of compounds against late-stage prostate cancer that is resistant to current treatments.

What was the impact on technology transfer?
We are continuing to work with NYU's Office of Therapeutics Alliances to develop a patent portfolio covering the technologies relevant to multivalent conjugates targeting the Androgen Receptor.

What was the impact on society beyond science and technology?
Nothing to report

5. CHANGES/PROBLEMS:

Nothing to Report

6. PRODUCTS:

Wang Y, Dehigaspitiya DC, Levine PM, Profit AA, Haugbro M, Imberg-Kazdan K, Logan SK, Kirshenbaum K, Garabedian MJ. Multivalent Peptoid Conjugates Which Overcome Enzalutamide Resistance in Prostate Cancer Cells. Cancer Res. Cancer Res. 2016 Sep 1;76(17):5124-32. doi: 10.1158/0008-5472.CAN-16-0385. Epub 2016 Aug 3.

Thomas, C., Fitting to overcome enzalutamide resistance, *Nature Reviews Urology* (2016) doi:10.1038/nrurol.2016.160 Published online 23 August 2016

7: PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

PIs: Name: Kent Kirshenbaum, PhD
Project Role: Initiating PI.
Nearest person month worked: 1
Contribution to Project:
Dr. Kirshenbaum conceived of the chemical platform and helped with the design and synthesis of the peptoid conjugates. He analyzed the experiments involving the chemical synthesis, and the cellular studies of MPC6 and its derivatives. He assisted in authoring the recent paper on MPC6 function and activity in prostate cancer
Funding Support: CDMRP

Name: Michael Garabedian, PhD.
Project Role: Partnering PI.
Nearest person month worked: 3
Contribution to Project:
Dr. Garabedian helped design and analyze the experiments involving the cell based proliferation assays and in vitro biochemical studies on MPC6 on AR. He also helped write the paper on MPC6 function and activity in prostate cancer.
Funding Support: CDMRP

Name: Kendall Nettles, PhD.
Project Role: Partnering PI.
Nearest person month worked: 3
Contribution to Project:
Dr. Nettles is performing the biophysical studies on the AR and MPC6. He is also involved in the DMPK studies.
Funding Support: CDMRP

Post docs and students

Name: Yu Wang, PhD
Project Role: Post doc
Nearest person month worked: 12
Contribution to Project:
Dr. Wang performed the experiments involving the cell based proliferation assays and in vitro biochemical studies on MPC6.
Funding Support: Prostate Cancer Foundation Young Investigator Awardee

Name: Amanda Kapser, PhD
Project Role: Post doc
Nearest person months worked: 6
Contribution to Project:
Dr. Kapser performed the synthesis of MPC6 and its derivatives.

Funding Support: NSF

Name: Dilani C. Dehigaspitiya, PhD
Project Role: Post doc
Nearest person months worked: 3
Contribution to Project:
Dr. Dehigaspitiya performed the synthesis of MPC6.
Funding Support: NSF

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report

What other organizations were involved as partners?
Nothing to Report

8 SPECIAL REPORTING REQUIREMENTS:

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to https://ers.amedd.army.mil for each unique award.

9 APPENDICES: see attached documents

Wang Y, Dehigaspitiya DC, Levine PM, Profit AA, Haugbro M, Imberg-Kazdan K, Logan SK, Kirshenbaum K, Garabedian MJ. Multivalent Peptoid Conjugates Which Overcome Enzalutamide Resistance in Prostate Cancer Cells. Cancer Res. 2016 Sep 1;76(17):5124-32. doi: 10.1158/0008-5472.CAN-16-0385. Epub 2016 Aug 3.

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Cancer Research

Multivalent Peptoid Conjugates Which Overcome Enzalutamide Resistance in Prostate Cancer Cells

Yu Wang[1], Dilani C. Dehigaspitiya[2], Paul M. Levine[2], Adam A. Profit[3], Michael Haugbro[2], Keren Imberg-Kazdan[4], Susan K. Logan[1,5], Kent Kirshenbaum[2], and Michael J. Garabedian[1,4]

Abstract

Development of resistance to antiandrogens for treating advanced prostate cancer is a growing concern and extends to recently developed therapeutics, including enzalutamide. Therefore, new strategies to block androgen receptor (AR) function in prostate cancer are required. Here, we report the characterization of a multivalent conjugate presenting two bioactive ethisterone ligands arrayed as spatially defined pendant groups on a peptoid oligomer. The conjugate, named Multivalent Peptoid Conjugate 6 (MPC6), suppressed the proliferation of multiple AR-expressing prostate cancer cell lines including those that failed to respond to enzalutamide and ARN509. The structure–activity relationships of MPC6 variants were evaluated, revealing that increased spacing between ethisterone moieties and changes in peptoid topology eliminated its antiproliferative effect, suggesting that both ethisterone ligand presentation and scaffold characteristics contribute to MPC6 activity. Mechanistically, MPC6 blocked AR coactivator–peptide interaction and prevented AR intermolecular interactions. Protease sensitivity assays suggested that the MPC6-bound AR induced a receptor conformation distinct from that of dihydrotestosterone- or enzalutamide-bound AR. Pharmacologic studies revealed that MPC6 was metabolically stable and displayed a low plasma clearance rate. Notably, MPC6 treatment reduced tumor growth and decreased Ki67 and AR expression in mouse xenograft models of enzalutamide-resistant LNCaP-abl cells. Thus, MPC6 represents a new class of compounds with the potential to combat treatment-resistant prostate cancer. *Cancer Res; 76(17); 1–9. ©2016 AACR.*

Introduction

The relationship between androgen receptor (AR) signaling and prostate cancer is well established. Prostate cancer cells are dependent on AR signaling, and for this reason, targeting AR is the mainstay of treatment for metastatic prostate cancer (1, 2). This usually involves either luteinizing hormone-releasing hormone (LHRH) agonists that prevent testicular androgen synthesis or AR antagonists, such as bicalutamide (Casodex), which block AR transcriptional activity (3). Although initial responses to these therapies are effective, they inevitably fail because castration-resistant prostate cancer cells emerge exhibiting enhanced AR activity (4). The realization that castration-resistant prostate cancers maintain their reliance on androgen signaling (5, 6) prompted the development of the second generation of antiandrogens, such as enzalutamide, which block AR action by inhibiting translocation of AR into the nucleus (7). Although enzalutamide represents a breakthrough in treatment of metastatic prostate cancer, patients who initially respond eventually acquire resistance (8). Finding effective new therapies for enzalutamide-resistant prostate cancer is an emerging clinical challenge (9).

We have developed a new approach for antagonizing AR function using multivalent peptidomimetic conjugates (MPC; reviewed in ref. 10). MPCs site-specifically display diverse bioactive ligands along an *N*-substituted glycine oligomer scaffold, termed a peptoid. Peptoids offer distinct advantages over other polymeric multivalent constructs because of the ability to control the exact number of conjugated ligands and their precise spacing, thereby enhancing affinity and specificity toward the desired target receptor (11). Unlike peptides, peptoids are resistant to proteases, thus providing improved pharmacologic stability. In addition, peptoids exhibit desirable water solubility and cell permeability characteristics (12). Previously, we developed a versatile synthetic platform that allows the modular design of diverse peptoid oligomer products with multiple spatially defined reactive side chains (13). We successfully ligated the AR targeting ligand ethisterone to the peptoid side chains to create a library of MPCs (13, 14). Among these, we demonstrated that one of the divalent multivalent peptoid conjugates, called MPC6, competed for androgen binding to the AR ligand binding domain (LBD) *in vitro*, and inhibited the proliferation and expression of AR target genes in androgen-independent LNCaP-abl cells (13, 14).

In this study, we determined the efficacy of MPC6 against a broad range of androgen-dependent and -independent prostate cancer lines, including enzalutamide-resistant variants, and tested the effectiveness of MPC6 *in vivo* in mouse xenograft models.

[1]Department of Urology, New York University School of Medicine, New York, New York. [2]Department of Chemistry, New York University, New York, New York. [3]York College, Institute for Macromolecular Assemblies and the Graduate Center of the City University of New York, Jamaica, New York. [4]Department of Microbiology, New York University School of Medicine, New York, New York. [5]Department of Biochemistry and Molecular Pharmacology, New York University School of Medicine, New York, New York.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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Using a chemical synthesis and screening platform, we also performed structure-function analyses to determine the impact of ligand spacing and conformational flexibility on MPC6 function. We conducted preliminary pharmacokinetic and pharmacodynamic studies and also interrogated the mechanism of MPC6 action. Our findings demonstrate that MPC6 is effective at blocking tumor growth in preclinical models of therapy-resistant prostate cancer.

Materials and Methods

Cell culture and treatments

LNCaP, 22Rv1, and PC-3 cells (ATCC), and C4-2 cells (UroCor) were cultured in RPMI-1640 with 10% FBS. LNCaP-abl cell (a gift from Dr. Zoran Culig, University of Innsbruck, Austria; ref. 15) and LNCaP-95 cell (a gift from Dr. John Isaacs and Dr. Jun Luo, John Hopkins University Baltimore, MD; ref. 16) were maintained in RPMI-1640 supplemented with 10% charcoal-stripped serum (CFBS). All cell lines were authenticated by short tandem repeat analysis and found to be mycoplasma free (Genetica). Cell lines were last authenticated January 5, 2016. Cycloheximide and 5α-Dihydrotestosterone (DHT) were purchased from Sigma-Aldrich. ARN509 and enzalutamide were purchased from Medkoo.

MPC synthesis

The synthesis procedure for 3-azido-1-aminopropane and linear peptoid scaffolds has been published previously (13). (*S*)-(+)-1-Methoxy-2-propylamine (Alfa Aesar) was used as the chiral amine to synthesize compounds MPC13 and MPC14. Each MPC candidate was purified to >95% by reverse phase analytical HPLC and verified by electrospray ionization mass spectrometry performed on an Agilent LCMSD Trap XCT mass spectrometer.

Cell proliferation assay

Cells were seeded in 96-well plates at a concentration of 3,000 cells per well. Cells were then treated with a single dose of the specified compound at the concentration indicated in the figure legends for 72 hours, and cell proliferation was measured by CyQUANT assay (Life Technologies) per manufacturer's protocol. MTT assays were also performed as described preciously (17).

Clonogenic assay

Single-cell suspensions of 1,000 cells were seeded into 100 mm culture dishes. Cells were treated with vehicle (0.1% DMSO), or 10 μmol/L enzalutamide, ARN509, or the MPC6 compounds. After 10 days, cells were fixed in 100% methanol for 15 minutes at room temperature and stained with 10% Giemsa (Gibco). Dishes were washed with water, and visible colonies containing approximately 50 or more cells were counted.

AR coactivator peptide binding assay

Coactivator peptide–AR interaction was evaluated using the LanthaScreen TR-FRET Androgen Receptor Coactivator Assay (Invitrogen) according to the manufacturer's instructions. Titrations of each treatment were plated in triplicate on a 384-well plate and were allowed to bind for approximately 4 hours before fluorescence polarization was measured. The fluorescence emission values at 520 nm and 495 nm, evaluated using excitation at 340 nm, were obtained using a SpectraMax M5 plate reader (Molecular Devices) and SoftMaxPro software. All data were processed using GraphPad in Prism.

Fluorescent-Two Hybrid assay for AR N/C interaction

Fluorescent-Two Hybrid (F2H) assay for live-cell analysis of AR N/C interactions was performed using the F2H-AR Kit (ChromoTek) as per the manufacturer's instructions. The AR-N-terminal-transcriptional domain (N) was fused to RFP and a DNA binding domain linked AR-C-terminal LBD (C) fused to GFP were cotransfected into F2H-BHK cells containing an arrayed cognate DNA binding element. Cells were treated with vehicle, 10 nmol/L DHT, or 10 μmol/L MPC6 for 24 hours. The AR N/C interactions were evaluated by confocal fluorescence microscopy (Zeiss).

Western blotting

Western blotting was performed as previously described (18). Mouse monoclonal anti-AR (441) (sc-7350) antibody was from Santa Cruz Biotechnology. Rabbit monoclonal anti–AR-V7 antibody (ab198394) was from Abcam. Mouse monoclonal anti–alpha-tubulin antibody (T9026) was from Sigma-Aldrich.

Quantitative real-time PCR

Total RNA was extracted by using a Qiagen RNeasy mini kit, and 1 μg of RNA was reverse transcribed into cDNA using random primers. cDNA amplification and quantification were done using the SYBR Green Taq Ready Mix (USB-Affymetrix) and the Life Technologies QS6 real time PCR detection system. *RPL19* gene expression was used as the control. Primer sequences are listed in Supplementary Table S2.

Protease sensitivity assay

LNCaP-abl cells were cultured in RPMI-1640 supplemented with 10% CFBS for 48 hours before being lysed with RIPA buffer. The lysate (250 μg) was incubated with vehicle control, 10 nmol/L DHT, 10 μmol/L MPC6, or 10 μmol/L enzalutamide at 4°C overnight. Samples were then digested with increasing concentrations of elastase (1 ng/mL, 10 ng/mL, 100 ng/mL, or 1 μg/mL) for 5 minutes at room temperature. The digestion products were loaded on SDS-PAGE gel, and AR peptides were detected by Western blot with anti-AR monoclonal antibody [AR (N441)] that recognizes an epitope between residues 299 and 315 of the human AR protein.

Microsomal stability assay

MPC6 (10 mmol/L) stock was diluted with water:acetonitrile (1:1) to a concentration of 100 μmol/L. Reaction mixture consisted of 2.5 μL MPC6 (100 μmol/L) and 165 μL of human or rat liver microsome (3.33 mg/mL). The reaction was initiated by adding 62 μL of NADPH and incubated at 37°C. After incubation, 25 μL of the sample was removed into 200 μL of acetonitrile to terminate the reaction at 0, 5, 10, 30, and 60 minutes. A 100 μL of supernatant was diluted with 100 μL of water and analyzed by LC-MS/MS. The assays were performed by GVK Biosciences.

Mouse xenograft studies

LNCaP-abl cells (1.5×10^7) were mixed with an equal volume of Matrigel (50 μl) and injected subcutaneously into the flank of 6-week-old NOD scid gamma (NSG) male mice. When tumors reached an average of 100 mm^3, mice were randomized into two groups of five and treated by intraperitoneal injection of DMSO or 50 mg/kg MPC6 twice a week. Tumor volume was measured twice weekly. After 3.5 weeks of treatment, mice were weighed and sacrificed. Xenograft studies were performed at the Memorial Sloan-Kettering Cancer Center and were approved by their

Institutional Animal Care and Use Committee. For immunohistochemistry, xenograft tumors were extracted and fixed in 4% (vol/vol) paraformaldehyde, embedded in paraffin, and sectioned. Tissue sections were prepared by NYUMC histopathology core and stained as previously described (19) using antibodies against AR (N-20; Santa Cruz Biotechnology; sc-816) and Ki67 (Cell Signaling Technology; cat # 9027).

Statistical analysis

Biochemical and cell-based assays were conducted at least three independent times, and the difference between two groups was determined by the Mann–Whitney test. The EC_{50} values were calculated using a nonlinear regression model. The tumor growth rate in the mouse xenograft experiment was calculated using a liner regression model.

Results

Ligand spacing and peptoid backbone architecture determined the efficacy of MPC6 on prostate cancer cell proliferation

We have previously described the synthesis of a linear peptoid oligomer conjugated with two steroidal ethisterone moieties at defined positions along the oligomer backbone that competed for androgen binding to the AR and showed antiproliferative effects against LNCaP-abl prostate cancer cells (13, 14). To examine whether increased spacing between the ethisterone moieties or changes to the peptoid backbone dynamics influenced activity, we synthesized a series of divalent ethisterone peptoid conjugates spaced by 8 (MPC6), 11 (MPC11), or 14 (MPC12) intervening monomers (Fig. 1A). We also created compounds MPC13 and MPC14 that possess similar monomer spacing to MPC6 and MPC11, but included an additional α-chiral, aliphatic side chain side within the intervening peptoid monomer, thus imparting chirality and constraining peptoid backbone flexibility (Fig. 1B; ref. 20). We tested these compounds in comparison with the AR antagonists enzalutamide and ARN509 for effects on LNCaP-abl cellular proliferation. Whereas LNCaP-abl cells were not growth inhibited by enzalutamide or ARN509, MPC6 significantly decreased the proliferation of LNCaP-abl cells (Fig. 1C). We previously demonstrated that MPC6 completed for DHT binding to AR using an *in vitro* ligand binding assay (13). By contrast, the divalent conjugates with increased spacing between ethisterone moieties, MPC11 and MPC12, had little effect on LNCaP-abl cell proliferation (Fig. 1C). Likewise, MPC13, the MPC6 analogue with structure-inducing intervening aliphatic side chain group, failed to elicit an antiproliferative response toward LNCaP-abl cells. These results largely paralleled the effects of different MPCs on their ability to antagonize AR-dependent transcriptional activation of an integrated AR-reporter gene in LNCaP cells (Supplementary Fig. S1). This suggests that both ligand spacing and conformational flexibility contribute to the antiproliferative effect of MPC6 toward an androgen-independent prostate cancer cell line.

Next, we determined the concentration of compound (MPC6, enzalutamide, ARN509, or ethisterone) required to inhibit the proliferation of LNCaP-abl cells by 50% (EC_{50}). MPC6 displayed an EC_{50} of approximately 3 μmol/L in LNCaP-abl cells. Neither enzalutamide nor ARN509 reached an EC_{50} even at the highest concentrations tested (100 μmol/L; Fig. 1D). The ethisterone ligand itself, as an isolated species, also failed to reach 50% inhibition at the maximal concentration tested (Fig. 1D). This suggests that multivalency plays an important role in the activity of MPC6. Because MPC6 significantly decreased the proliferation of a therapy-resistant prostate cancer cell line, we focused on this candidate in our subsequent studies.

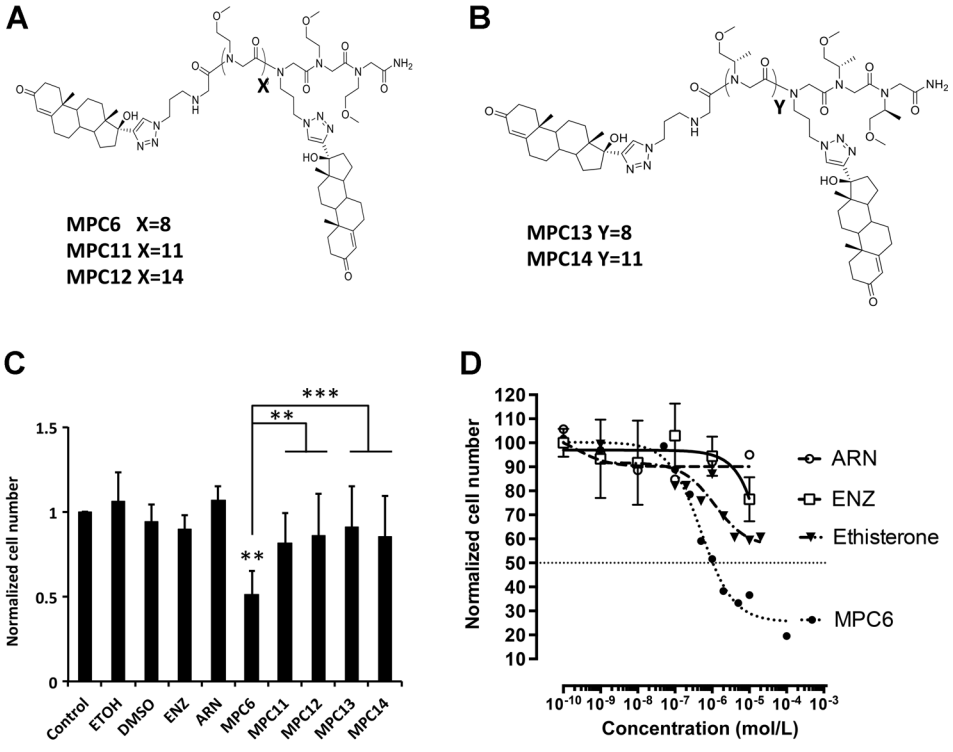


Figure 1.
MPCs affect the proliferation of LNCaP-abl cells. **A,** chemical structures of linear divalent ethisterone–peptoid conjugates MPC6, MPC11, and MPC12, spaced by 8, 11, and 14 monomers, respectively. **B,** chemical structures of divalent ethisterone–peptoid conjugates MPC14 and MPC15 with a methyl group on peptoid backbone. **C,** LNCaP-abl cells were treated with 10 μmol/L of vehicle [ethanol (EtOH) or DMSO], enzalutamide (ENZ), ARN509, or MPCs for 72 hours, and cell proliferation was measured. The error bar represents SD from six independent experiments. **, $P < 0.01$; ***, $P < 0.001$. **D,** LNCaP-abl cells were treated with the indicated compounds and concentrations. After 72 hours, cell proliferation was measured and the EC_{50} calculated. Each point represents a mean value of three independent experiments. Error bars, SD.

MPC6 inhibited the proliferation of AR-expressing prostate cancer cells lines representing a spectrum of disease states

We next compared the ability of MPC6 and enzalutamide to inhibit the proliferation of a variety of prostate cancer cell lines that represent a spectrum of disease states, including androgen-dependent (LNCaP), androgen-independent (LNCaP-C4-2, LNCaP-abl, 22Rv1, LNCaP-95), and AR-deficient (PC3) classes. AR-negative PC3 cells did not respond to MPC6 (Fig. 2A), confirming our previous finding that antiproliferative effects were not attributable to general cellular toxicity (13). 22Rv1 cells, harboring both AR splice variants and full-length AR with a H875Y mutation in the LBD, also did not respond to MPC6 treatment (Fig. 2A). Importantly, MPC6 treatment decreased the proliferation of androgen-dependent LNCaP cells, bicalutamide-resistant LNCaP-C4-2 cells (18), along with androgen-independent and enzalutamide-resistant LNCaP-abl (15) and LNCaP-95 (16) cells. LNCaP-95 cells harbor both full-length AR (without the H875Y mutation) and the AR-V7 splice variant frequently observed in patients with enzalutamide resistance (Fig. 2B; refs. 16, 21, 22).

Comparison of the IC_{50} of MPC6 and enzalutamide revealed a lower concentration of MPC6 was required to inhibit the proliferation of LNCaP (MPC6 = 4 μmol/L; enzalutamide = 19 μmol/L),

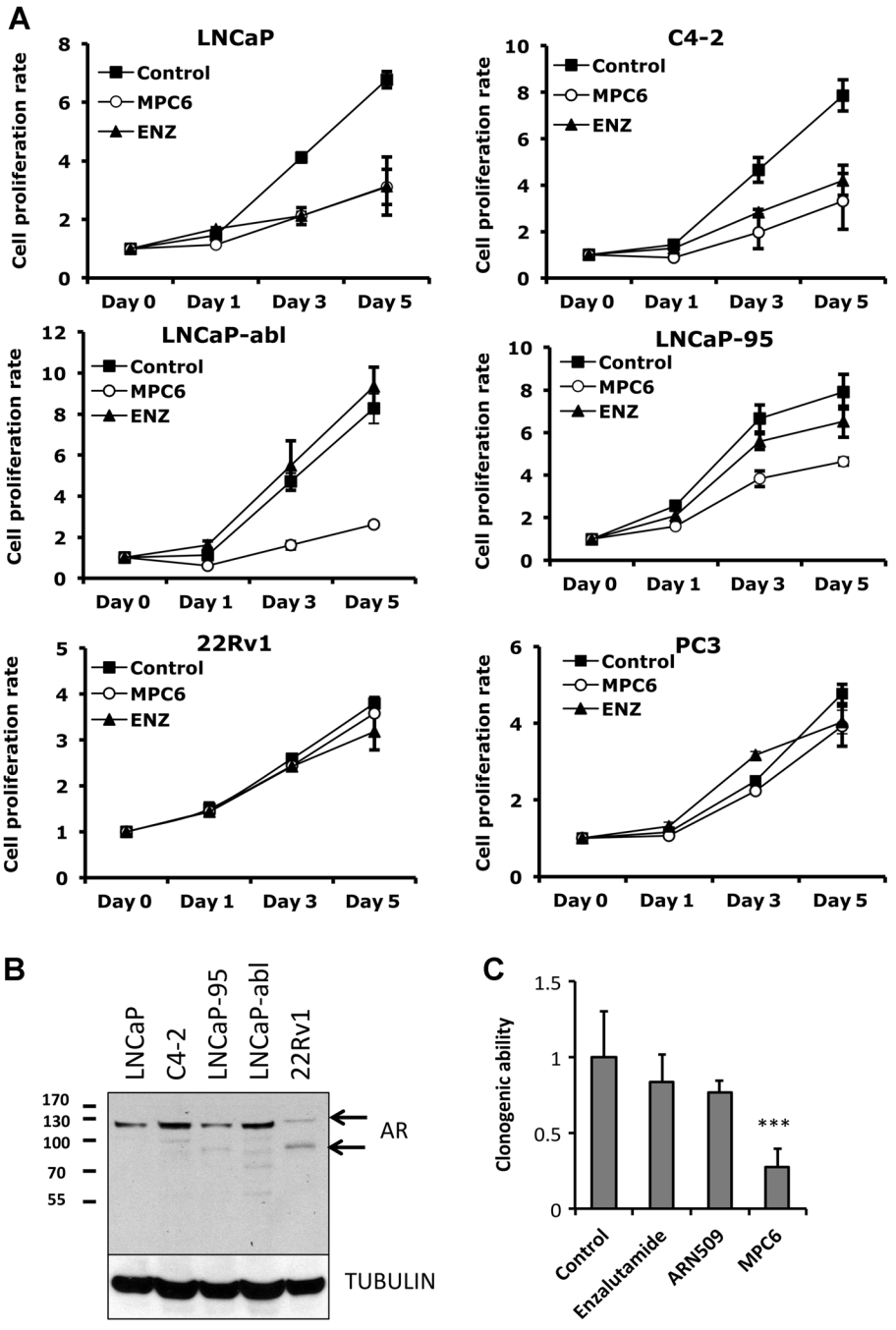


Figure 2.
MPC6 inhibits proliferation of multiple AR-expressing prostate cancer cell lines. **A,** AR-expressing prostate cancer cell lines (LNCaP, LNCaP-C4-2, LNCaP-abl, LNCaP-95, and 22Rv1) and AR-negative PC3 cells were treated with control or a single dose of 10 μmol/L MPC6 for 5 days, and cell growth was measured. **B,** Western blot of AR from indicated prostate cancer cell lines. **C,** colony formation in LNCaP-abl cells, treated with vehicle, 10 μmol/L enzalutamide, 10 μmol/L ARN509, or 10 μmol/L MPC6, was measured by a clonogenic assay. ***, $P = 0.0003$.

LNCaP-C4-2 (MPC6 = 5.6 μmol/L; enzalutamide = 18.5 μmol/L), LNCaP-abl (MPC6 = 3.2 μmol/L; enzalutamide = not reached), and LNCaP-95 cells (MPC6 = 6.7 μmol/L; enzalutamide = not reached; Supplementary Table S1). AR protein abundance was largely unaffected by MPC6 treatment after 48 hours in AR-expressing prostate cancer cells (Supplementary Fig. S2).

We also tested whether MPC6 could affect the proliferation of non–prostate cancer cell lines. Whereas the uterine cancer cell line RL95-2 (23), which expresses AR (24), was growth inhibited upon MPC6 treatment, the colon cancer cell line HCT116, which does not express AR (25), was not growth inhibited by MPC6 (Supplementary Fig. S3). Thus, MPC6 treatment inhibited the proliferation of a wide range of AR-expressing prostate cancer cell lines, and also repressed the proliferation of a uterine cancer cell line.

We also used LNCaP-abl cells to compare the effect of MPC6, enzalutamide, or ARN509 treatment on colony formation from single cells using a clonogenic assay (26). This assay measures the survival and replicative potential of individual cancer cells and is a surrogate for how treatment with compounds affects metastatic growth. Neither enzalutamide nor ARN509 could effectively prevent colony formation in LNCaP-abl cells, whereas MPC6 inhibited colony formation by over 70% relative to vehicle-treated cells (Fig. 2C). This was not a result of cell death by MPC6 but rather inhibition of proliferation that decreased the formation of colonies (14). Thus, MPC6 reduced the growth of single LNCaP-abl cells into colonies, whereas the next-generation anti-androgen compounds could not.

MPC6 blocks AR–coactivator peptide interaction and reduces AR abundance without affecting AR nuclear localization

To elucidate the mechanism of MPC6 action on AR activity, we tested whether MPC6 treatment affected nuclear localization in LNCaP-abl cells using a cellular fractionation approach. In LNCaP-abl cells, AR was evident in both the cytoplasm and nucleus in the absence of androgen, followed by increased AR accumulation within the nucleus upon androgen treatment (Fig. 3A; compare lanes 1 and 2 with 7 and 8). Consistent with its reported mechanism of action, enzalutamide reduced AR nuclear localization in both the absence and presence of added androgens (Fig. 3A; compare lanes 8 to 12 and lanes 2 to 6). By contrast, in the absence of androgen, MPC6 treatment resulted in a markedly decreased cytoplasmic AR protein levels with effects on nuclear AR levels compared with control (Fig. 3A; compare lanes 7 and 9). Interestingly, the presence of androgens abolished the effect of MPC6 on the reduction of cytoplasmic AR protein (Fig. 3A compare lanes 3 to 9). Thus, unlike enzalutamide, the major impact of MPC6 is not to antagonize AR by preventing its nuclear accumulation (Fig. 3A).

We next evaluated whether MPC6 blocked the interaction between AR and coactivator proteins. For this, we used an *in vitro* time resolved fluorescence resonance energy transfer (TR-FRET) assay. In this assay, the interaction between the AR-LBD and an FxxLF coactivator peptide was monitored in the presence of ligand by a TR-FRET signal between a terbium-labeled AR and a fluorescein-labeled coactivator peptide (27). As expected, DHT induced a dose-dependent interaction between AR and the FxxLF-motif containing peptide, indicative of coactivator binding (Fig. 3B). By contrast, MPC6 prevented agonist-induced interaction between AR and the coactivator peptide (Fig. 3B). These data suggest that MPC6 induced an AR conformation that was incompatible with coactivator binding.

To test the effect of MPC6 on AR conformation, we probed protease sensitivity differences between AR bound to MPC6, DHT, and enzalutamide. LNCaP-abl cell lysates were incubated with DMSO (control), 10 nmol/L DHT, 10 μmol/L MPC6, or 10 μmol/L enzalutamide overnight at 4°C to allow ligand binding. Lysates were then digested with increasing amounts of elastase for 5 minutes at room temperature. Elastase cleaves at the carboxyl side of alanine and glycine and different digestion patterns represent distinct protein conformations and exposed surfaces. AR peptide fragments were visualized by Western blot analysis. MPC6 produced a digestion pattern of AR similar to that of the unbound AR control (Fig. 3C; compare lanes 2–5 with lanes 7–10), with the exception of two prominent protected fragments at the lowest and highest elastase concentrations in the control compared with MPC6-bound AR (Fig. 3C; dark arrows in lanes 2 and 5 compared with lanes 7 and 10). An additional stable fragment was observed in the AR–DHT complex that was not observed in MPC6 and enzalutamide bound AR (arrowhead, compare lanes 4 and 14). This suggested that the MPC6-bound AR induced a conformation distinct from the enzalutamide-bound AR.

To further interrogate MPC6-induced AR conformational changes, we used a fluorescent two-hybrid assay for live-cell analysis of AR interdomain interaction (28). This assay uses an AR N-terminal activation domain-RFP fusion protein and an AR C-terminal LBD linked to GFP and fused to a heterologous DNA binding domain to evaluate interaction in a cell harboring an arrayed DNA binding site. In this assay, androgen agonists trigger an interaction between the AR amino- and carboxy-terminal (N/C) domains that is visualized as the red spot in the nucleus indicative of recruitment of the AR N-terminus to the agonist bound AR C-terminal LBD tethered to the DNA array in the cell (29). In androgen-free media, the red spot in the nucleus is not observed, whereas the AR C-terminal LBD-GFP bound to DNA appears as a bright green spot (Fig. 3D; top). In cells cultured in complete media that contain androgenic compounds, the AR-ligand binding domain and AR-N-terminal activation domain colocalized in the cell nucleus under basal vehicle treatment, which is also observed upon DHT treatment (Fig. 3D). By contrast, enzalutamide, ARN509, and MPC6 treatment suppressed AR interdomain interaction (Fig. 3D). It is interesting to note that the level of AR LBD in the nucleus was reduced in enzalutamide and ARN509-treated samples as shown by an overall decrease in GFP signal, whereas the GFP signal was retained in MCP6-treated cells. This is consistent with our subcellular fraction study (Fig. 3A) and suggests that MPC6 inhibits AR activity through a different mechanism than enzalutamide and ARN509. Thus, the binding of MPC6 suppressed the interaction between AR N/C termini.

MPC6 reduced AR-V7 expression and suppressed AR-V7 target gene expression

AR alternative splicing and expression of AR-variant 7 (AR-V7) is associated with enzalutamide resistance and poor prognosis (8, 21). To investigate the impact of MPC6 on *AR-V7* expression in enzalutamide-resistant prostate cancer, we treated LNCaP-95 with MPC6 or enzalutamide. MPC6 significantly decreased *AR-V7* mRNA expression in LNCaP-95 cells (Fig. 4A). This was accompanied by decreased AR-V7 and concomitant increase in full-length AR protein abundance (Fig. 4B). In contrast, enzalutamide increased *AR-V7* mRNA expression and protein levels in LNCaP-

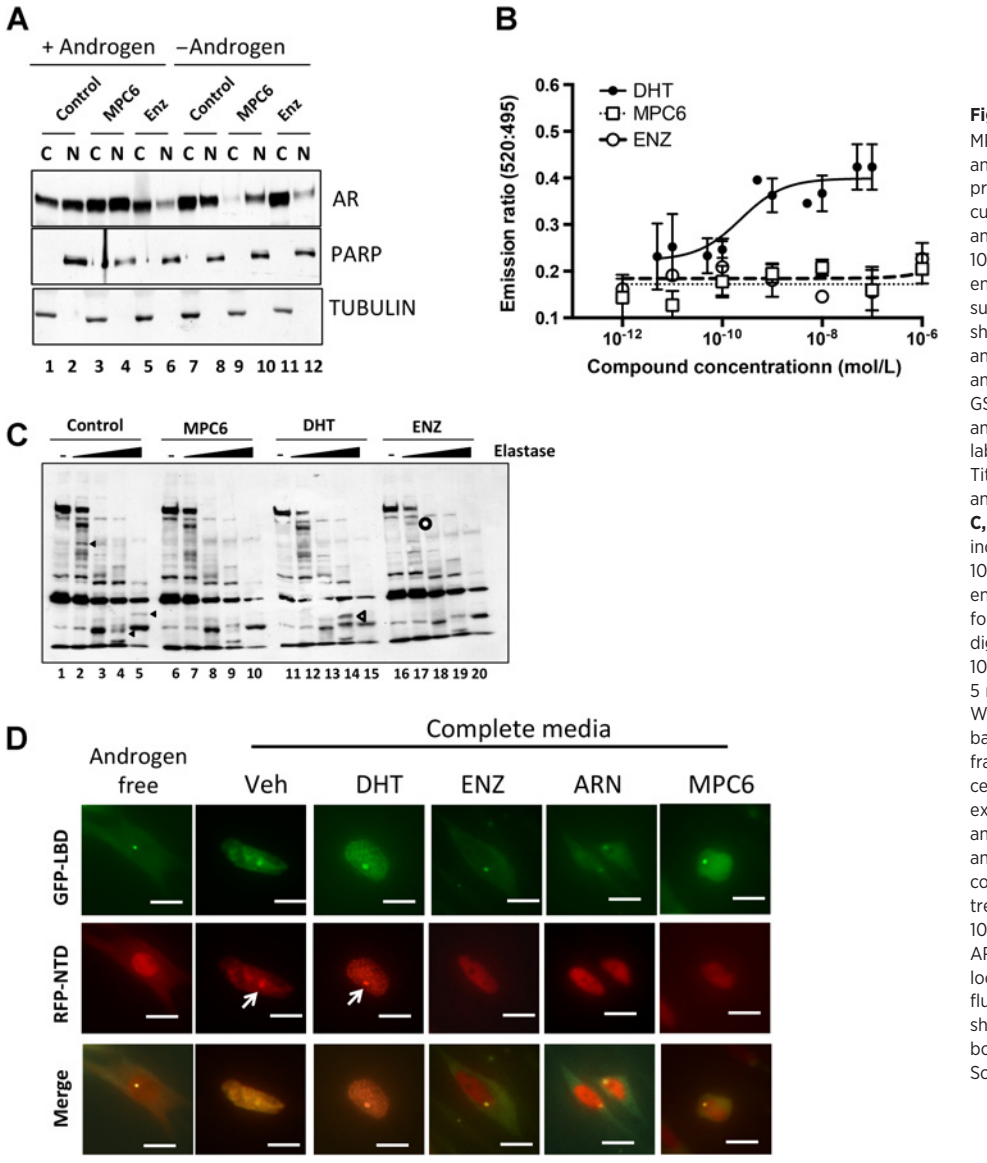


Figure 3.
MPC6 blocked AR-coactivator binding, and AR interdomain interaction in prostate cancer cells. **A,** LNCaP-abl cells cultured in the presence or absence of androgen were treated with vehicle, 10 μmol/L MPC6, or 10 μmol/L enzalutamide overnight before subcellular fractionation. Western blot shows AR expression in cytoplasm (C) and nucleus (N). **B,** *in vitro* TR-FRET analysis of the interaction between GST-tagged AR-LBD, terbium-labeled anti-GST antibody, and fluorescein-labeled AR FxxLF coactivator peptide. Titration of DHT, enzalutamide (ENZ), and MPC6 for 10 nmol/L DHT bound AR. **C,** LNCaP-abl cell lysates were incubated with control, 10 nmol/L DHT, 10 μmol/L MPC6, or 10 μmol/L enzalutamide overnight at 4°C to allow for ligand binding. Lysates were digested with 1 ng/mL, 10 ng/mL, 100 ng/mL, or 1 μg/mL elastase for 5 minutes at room temperature and Western blot performed for AR. Marked bands indicate differences in AR fragments among treatments. **D,** BHK cells were cotransfected with expression plasmids for AR-LBD-GPF and AR-NTD-RFP, and cultured in androgen-free or standard media containing androgens. Cells were treated with vehicle or 10 nmol/L DHT, 10 μmol/L enzalutamide, 10 μmol/L ARN509, or 10 μmol/L MPC6, and localization was examined using fluorescent microscopy. White arrows show interaction of AR-NTD with DNA-bound AR-LBD. Magnification, ×400. Scale bar, 3 μm.

95 cells (Fig. 4A and B). A previous study has shown that AR-V7 regulates a distinct set of target genes relative to the full-length AR, including *UBEC2* (16). Therefore, we evaluated *UBE2C* mRNA expression upon MPC6 or enzalutamide treatment. Whereas enzalutamide treatment significantly increased *UBE2C* mRNA expression, MPC6 suppressed *UBE2C* transcription (Fig. 4C). This suggests that MPC6 can reduce AR-V7 expression and function in LNCaP-95 cells.

MPC6 exhibits favorable pharmacologic properties

Our data demonstrated that MPC6 effectively suppressed the proliferation of a subset of enzalutamide- and ARN509-resistant prostate cancer cells lines. To examine the pharmacologic properties of MPC6, we conducted PK/PD studies of metabolic stability in microsome assays. Microsomes from human and rat liver cells were used to measure the rate of MPC6 metabolism compared with control compounds with high (verapamil, $T^1/_2 \sim 10$ minutes), medium (propranolol, $T^1/_2 \sim 55$ minutes), and low (phenacetin, $T^1/_2 \sim 80$ minutes) clearance rates (Table 1). MPC6 had a low clearance rate in both human and rat liver microsomes with a half-life of 9 and 30 hours, respectively (Table 1). Its intrinsic clearance rate (Cl_{int}) was 1.5 μL/min/mg in human and 0.5 μL/min/mg in rodent, indicating a favorable pharmacokinetic profile (Table 1).

We also examined MPC6 bioavailability. We used male rats and tested MPC6 absorbance and plasma concentration after oral and i.v. administration over a 24-hour time course. Oral delivery of MPC6 at 10 mg/kg achieved a maximum plasma concentration of 100 ng/mL within 30 minutes, which then decreased to the lowest level of detection (3.4 ng/mL) after 4 hours (Supplementary Fig. S4). I.v. injection of MPC6 achieved a maximum plasma concentration of 5,000 ng/mL within 5 minutes and maintained a steady concentration (20 ng/mL) for at least 24 hours (Supplementary Fig. S4). Thus, MCP6 was bioavailable via both oral and

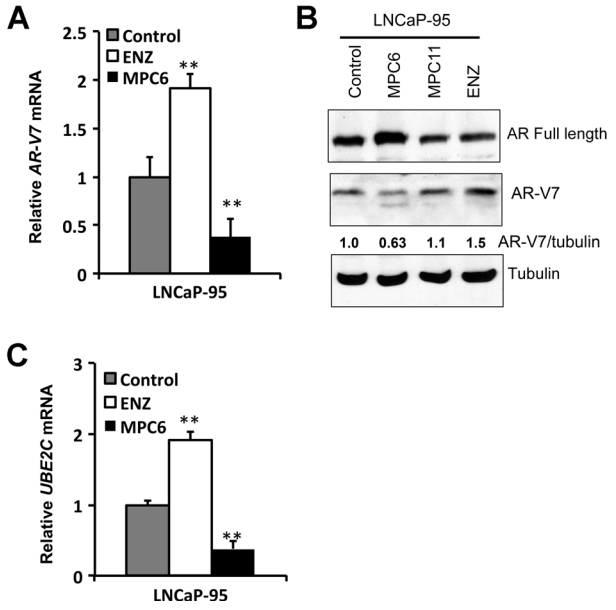


Figure 4.

MPC6 decreased AR-V7 expression and AR-V7 target gene transcription in prostate cancer cells. **A,** LNCaP-95 cells were treated with vehicle, 10 μmol/L MPC6, or 10 μmol/L enzalutamide (ENZ) for 48 hours. Total RNA was extracted, reverse transcribed, and qRT-PCR was performed to assess *AR-V7* mRNA expression relative to RPL19. **, $P < 0.01$. **B,** LNCaP-95 cells were treated with vehicle, 10 μmol/L MPC6, 10 μmol/L MPC11, or 10 μmol/L enzalutamide for 48 hours. AR full-length and AR-V7 protein levels were evaluated by Western blot. Numbers indicate the AR-V7 protein abundance relative to tubulin. **C,** LNCaP-95 cells were treated with vehicle, 10 μmol/L MPC6, or 10 μmol/L enzalutamide for 48 hours. Total RNA was extracted, reverse transcribed, and qRT-PCR performed to assess *UBE2C* mRNA expression. **, $P < 0.01$.

i.v. routes, although i.v. administration resulted in a greater and more sustained steady-state plasma level.

MPC6 suppresses growth of prostate cancer xenografts

To test whether MPC6 suppressed prostate cancer cell proliferation *in vivo,* we used LNCaP-abl xenografts in immune compromised mice. Mice were treated with vehicle or MPC6 twice a week at 50 mg/kg (MTD of MPC6 in mice is >150 mg/kg; data not shown) for 3.5 weeks, and tumor volume was measured over time. After 3.5 weeks of treatment, MPC6 significantly suppressed LNCaP-abl tumor growth ($P = 0.004$; Fig. 5A). MPC6 at 50 mg/kg dose was well tolerated as mice did not display any significant change in body weight (Supplementary Fig. S5). Histologic examination of the tumors revealed dramatically reduced cellularity in the MPC6-treated compared with vehicle-treated tumors (Fig. 5A). Immunohistochemical staining of the MPC6-treated compared with control tumors showed decreased AR protein ($P = 0.003$) and Ki67 levels ($P < 0.0001$; Fig. 5B). These results suggested that MPC6 reduced prostate tumor growth by targeting AR.

Discussion

We have characterized a divalent ethisterone conjugate MPC6 that blocked the proliferation of a variety of AR-expressing androgen-dependent and androgen-independent prostate cancer cell lines. MPC6 also showed efficacy *in vivo* against LNCaP-abl tumor xenografts. MPC6 was metabolically stable and blocked the interaction of AR with a coactivator peptide and interfered with AR interdomain interaction in LNCaP-abl cells. Our previous findings showed that MPC6 competed for androgen binding to the AR LBD *in vitro* (13) and inhibited AR target genes that promoted the cell cycle in LNCaP-abl cells (14). MPC6, the divalent conjugate in which the steroidal groups are arrayed with eight intervening monomers, appeared to have the most potent antiproliferative effect as shorter spacing (two or four intervening monomers, see ref. 13) or longer spacing (11 or 14 intervening monomers, this study) between the ethisterone ligands diminished compound activities. The architecture of the peptoid scaffold also contributed to the antiproliferative activity of MPC6. Therefore, both ligand spacing and the conformational flexibility of peptoid backbone play important roles in MPC6 efficacy. Currently, it is unclear why particular peptoid oligomer chain lengths and monomer types are required to achieve maximal efficacy. However, we suggest that the ligand spacing and backbone topology of MPC6 can promote a conformation upon binding to the AR that is incompatible with coactivator and AR interdomain interactions. Flexibility of the peptoid backbone may also be required for productive binding interactions that could be constrained by structure-inducing side chain groups. Detailed structural studies will be needed to reveal the requirements for ligand spacing and peptoid backbone participation in MPC6 activity.

Multivalency has become increasingly important as a therapeutic strategy (30–32). Multivalent conjugates have several advantages over traditional small molecules. Multivalent

Table 1. MPC6 stability in liver microsomes

Compound name	Species	Half-life *minute [hour]*	Cl_{int} *(μL/min/mg)*	% Rem *@ 60 minutes*	Clearance[a] classification
	Human				
MPC6		551.8 [9.1 h]	1.5	88.6	Low
Propranolol		55.1	12.6	46.5	Medium
Verapamil		9.8	70.5	1.5	High
	Rat				
MPC6		1796.6 [29.9 h]	0.52	94.5	Low
Propranolol		80.2	8.6	58.7	Medium
Verapamil		8.3	83.3	0.53	High

NOTE: Cl_{int}, intrinsic clearance rate; % Rem, percent remaining.

[a]Classification criteria:

Human: Low, <10.82 μL/min/mg protein; Medium, 10.82–58.8 μL/min/mg protein; High, >58.8 μL/min/mg protein.

Rat: Low, <16.67 μL/min/mg protein; Medium, 16.67–90.74 μL/min/mg protein; High, >90.74 μL/min/mg protein.

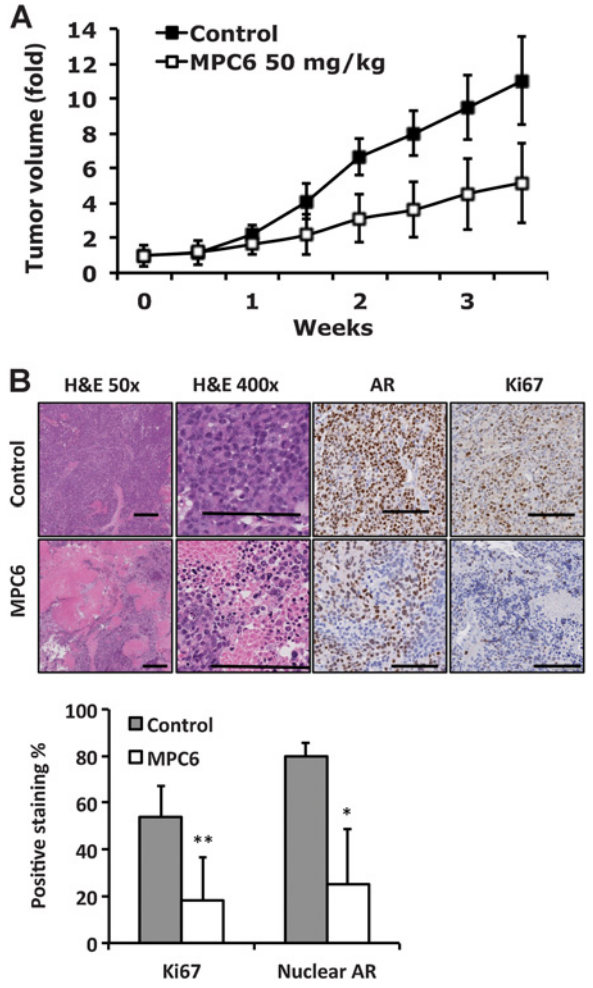


Figure 5.

MPC6 suppressed LNCaP-abl xenograft growth. **A,** nude mice bearing LNCaP-abl xenografts were treated with vehicle ($n = 5$) or 50 mg/kg MPC6 ($n = 5$) twice a week for 3.5 weeks. Tumor volumes were measured on the indicated days ($P = 0.004$). **B,** tumors from mouse xenografts were removed and subjected to hematoxylin and eosin (H&E) staining and immunohistochemistry for AR and Ki67. Scale bar, 150 μm. Positive nuclear AR expression and Ki67 staining in MPC6-treated groups ($n = 5$) were quantified and compared with that of control group ($n = 5$). *, $P = 0.003$; **, $P < 0.0001$.

conjugates possess increased activity toward their targets as a result of enhanced avidity compared with that of monovalent ligands (11). In fact, the divalent ethisterone peptoid conjugate MPC6 effectively inhibited prostate cancer cell proliferation at a much lower dose than the unconjugated ethisterone (Fig. 2D). Consistent with the alterations in the backbone reducing MPC6 efficacy, we also suggest that the peptoid backbone participates in perturbing the AR conformation to reduce AR transcriptional activity.

MPC6 also inhibited the proliferation of LNCaP-95 cells, which expressed both full-length AR and the AR-V7 splice variant, and reduced AR-V7 expression. Previous studies have demonstrated that treatment of LNCaP-95 cells with enzalutamide stimulated the expression of the AR-V7 splice variant (21, 22), thus promoting enzalutamide resistance. This is likely as a consequence of AR exclusion from the nucleus and subsequent effects on splicing of the *AR* mRNA. In fact, AR has been shown to interact with several splicing factors in the nucleus, and androgen-deprivation promotes the recruitment of numerous splicing factors to the 3′ splice site of the AR pre-mRNA to enhance the *AR-V7* transcript (33). We speculate that MPC6, by virtue of its ability to antagonize AR without removing it from the nucleus, would maintain accurate splicing and prevent the accumulation of the AR-V7 splice variant. Whether enzalutamide promotes, and MPC6 suppresses, AR-V7 splice site enhancement through alterations in splicing factor recruitment remains an open question.

The proliferation of 22Rv1 cells, by contrast, was not inhibited by MPC6. Although 22Rv1 cells expressed a number of AR splice variants as a result of a duplication of exon 3 (34), the cells also express the full-length AR with a H875Y mutation in the LBD (35). This might preclude MPC6 binding to AR and explain the lack of responsiveness to MPC6 treatment by 22Rv1 cells. Importantly, MPC6 reduced the proliferation of both androgen-dependent LNCaP cells, and androgen-independent cell lines including LNCaP-abl and LNCaP-95 cells that were refractory to enzalutamide treatment. Thus, MPCs represent a promising new class of compounds with the potential to block the proliferation of therapy-resistant prostate cancer cells expressing promiscuous and wild-type AR.

Disclosure of Potential Conflicts of Interest

K. Kirshenbaum has ownership interest, including a patent on MPCs. No potential conflicts of interest were disclosed by the other authors.

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Conception and design: Y. Wang, D.C. Dehigaspitiya, P.M. Levine, S.K. Logan, K. Kirshenbaum, M.J. Garabedian

Development of methodology: Y. Wang, P.M. Levine, K. Imberg-Kazdan

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y. Wang, P.M. Levine, K. Imberg-Kazdan, K. Kirshenbaum

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y. Wang, K. Imberg-Kazdan, S.K. Logan, K. Kirshenbaum, M.J. Garabedian

Writing, review, and/or revision of the manuscript: Y. Wang, D.C. Dehigaspitiya, P.M. Levine, S.K. Logan, K. Kirshenbaum, M.J. Garabedian

Other (synthesis of peptoid conjugates): D.C. Dehigaspitiya, P.M. Levine, A.A. Profit, M. Haugbro

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PROSTATE CANCER

Fitting to overcome enzalutamide resistance

> …MPC6 induced a distinct AR conformation and blocked interaction with AR coactivator peptides…

A new ethisterone peptoid conjugate that blocks androgen receptor (AR) function can overcome enzalutamide resistance in prostate cancer models. The report, published in *Cancer Research*, describes the biological evaluation of a new set of multivalent peptoid conjugates (MPCs) and highlights the potential of new compounds based on innovative medicinal chemistry designs against diseases that are resistant to current treatments.

MPCs are sequence-specific oligomers that are synthetic variants of peptides. The peptoids are composed of *N*-substituted glycine monomers, so that the side chains are appended from the backbone nitrogen atoms. "Peptoids enable precise positioning of multiple ligands along the oligomer backbone," explain Michael Garabedian and Kent Kirshenbaum from New York University, senior authors of the study. "This multivalent presentation of the ligand can improve ligand–receptor interaction, owing to increased local ligand concentration, and can impart new pharmacological ligand properties, owing to the oligomeric set-up."

The team had previously designed a series of MPCs containing ethisterone ligands and investigated their mechanism of action as AR antagonists. In the current report, they performed structure–activity analyses on the most promising molecule MPC6 by comparing its properties with those of compounds with wider spacing between ethisterone ligands or modified peptoid backbone flexibility. Only MPC6 inhibited growth of enzalutamide-resistant LNCaP cells and comparison with ethisterone alone demonstrated that multivalency of the ligand was required for activity at applicable concentrations. Further *in vitro* experiments showed that binding of MPC6 induced a distinct AR conformation and blocked interaction with AR coactivator peptides, possibly illustrating the reason for the lack in activity of related compounds that have a different chemical structure.

In contrast to enzalutamide, MPC6 also inhibited growth of cells expressing the AR splice variant AR-V7 and the expression of AR-V7 itself. MPC6 treatment of *in vivo* xenografts of the enzalutamide-resistant LNCaP cells showed significantly reduced tumour growth compared with vehicle administration.

"Next, we will perform additional medicinal chemistry to enhance MPC6 activity, working in collaboration to determine the X-ray crystal structure of MPC6 bound to AR to rationally inform modifications," Garabedian concludes. "Our ultimate goal is to move this approach into clinical application for patients who are resistant to enzalutamide therapy."

Clemens Thoma

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